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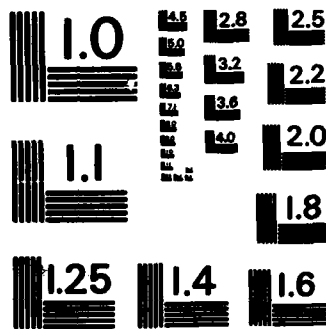
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ACUTE DERMAL TOXICITY OF CHF 1, CHR 2 AND SALINE IN RABBITS

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and

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DIVISION OF RESEARCH SUPPORT

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SEPTEMBER 1982

Toxicology Series 32

LETTERMAN ARMY INSTITUTE OF RESEARCH  
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

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**Acute Dermal Toxicity of CHF 1, CHF 2, and Saline--Hanes et al  
Toxicology Series 32**

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*Paul Marshall J. 8 Apr 82*  
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)		
<p>The acute dermal toxicity potential of CHF1 AND CHR2 was determined in rabbits using abraded skin sites and plastic covering over the exposed areas for 24 hrs. No animals died. Skin irritation was greatest in the flank regions, probably due to gravity accumulation and pressure due to skin folds and adhesive bandages. These compounds should be exposed to further toxicological testing for human use potential.</p>		

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# ABSTRACT

The acute dermal toxicity potential of CHF1 and CHF2 was determined in rabbits using abraded skin sites and plastic covering over the exposed areas for 24 hours. No animals died. Skin irritation was greatest in the flank regions, probably due to gravity accumulation and pressure due to skin folds and adhesive bandage. These compounds should be exposed to further toxicological testing for human use potential.

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## **PREFACE**

**TYPE REPORT:** Acute Dermal Toxicity GLP Report

**TESTING FACILITY:** Letterman Army Institute of Research  
Presidio of San Francisco, CA 94129

**SPONSOR:** Letterman Army Institute of Research  
Presidio of San Francisco, CA 94192

**PROJECT/WORK UNIT/APC:** Prevention of Military Disease Hazards  
3M16770A871, APC FL07

**GLP STUDY NUMBER:** 81009

**STUDY DIRECTOR:** COL John T. Fruin, D.V.M., PhD, VC, Diplomate of  
American College of Veterinary Preventive Medicine

**PRINCIPAL INVESTIGATOR:** CPT Martha A. Hanes, DVM, VC

**PATHOLOGIST:** LTC Paul Mellick, DVM, PhD, VC, Diplomate of American  
College of Pathologists

**REPORT AND DATA MANAGEMENT:** A copy of the final report, study protocol,  
and retired SOPs will be retained in the  
LAIR Archives.

**TEST SUBSTANCES:** A. CHR1 - formulation of 50% N,N-diethyl-m-toluamide  
(m-DEET) in 25% Dow Corning 200 Fluid and 25%  
isopropyl alcohol.

B. CHR2 - n-octyl glutarimide

**INCLUSIVE STUDY DATES:** 29 Apr - 22 Jun 81

**OBJECTIVE:** The purpose of this study was to determine the acute  
dermal toxicity potential of the test substances listed  
above.

#### ACKNOWLEDGMENTS

The authors wish to thank SSG Lance White; SP4 Thomas Kellner, BA; SP4 Lawrence Mullen, BS; SP4 Evelyn Zimmerman; Carolyn Lewis, MS; and John Dacey for their assistance in performing the research. The authors also wish to thank William Riefenrath, PhD, for providing the chemical and Louis Rutledge for the background information.



SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, believe the study number 81009 described in this report to be scientifically sound and the results in this report and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Non-Clinical Laboratory Studies, outlined by the Food and Drug Administration.

*John T. Fruin 23 Apr 82*  
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DEPARTMENT OF THE ARMY  
LETTERMAN ARMY INSTITUTE OF RESEARCH  
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO  
ATTENTION OF:

SGRD-ULZ-QA

4 June 1982

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 81009 the following inspections were made:

13 May 81  
14 May 81  
27 May 81  
8 Jun 81  
9 Jun 81  
22 Jun 81

The report and raw data for this study were audited on 1 Jun 82.

Routine inspections with no adverse findings are reported quarterly, thus these inspections were also included in the 1 Jul 81 report to management and the Study Director.

JOHN C. JOHNSON  
CPT, MS  
Quality Assurance Officer

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## ACUTE DERMAL TOXICITY OF CHF 1, CHR 2 AND SALINE IN RABBITS--Hanes et al

The goal of the insect repellent program is to develop better insect repellents for the protection of soldiers from insects and insect-borne diseases in the field. In the last several years the Division of Cutaneous Hazards, Letterman Army Institute of Research (LAIR), had tested a large number of chemical compounds, submitted by SRI International, the U.S. Department of Agriculture (USDA), and private industry, against a variety of mosquitoes, sand flies, fleas, bus, ticks, and mites in animals and in vitro test systems. Several of these materials have shown sufficient repellent activity and persistence on the skin of animals to warrant consideration for use in lieu of, or in conjunction with, the current troop-issue insect repellent, 71.25% N,N-diethyl-m-toluamide (m-DEET) in ethanol. The Division of Cutaneous Hazards has also evaluated a number of new formulations of m-DEET prepared at LAIR or submitted by private industry. Several of these new formulations have been more persistent than the current troop-issue repellent in tests on animals.

### Toxicity Testing Repellent Program

It is now planned to test the best of the new compounds and formulations on human volunteers to confirm the results that have been obtained in the in vitro and animal tests and to evaluate their performance under conditions of actual use. Before this can be done, it is necessary to obtain certain toxicity data on each compound or formulation to insure that it is safe for application to the skin. The toxicity tests required for registration of a new insect repellent are prescribed by the Environmental Protection Agency (EPA). The basic animal toxicity tests required for experimental use of the new compounds and formulations on human volunteers are prescribed by the LAIR and USAMRDC Human Use Committees. An acute dermal toxicity ( $LD_{50}$ ) test is one of the animal toxicity tests of CHF1 and CHR2 requested by the Division of Cutaneous Hazards so that the formulation could be considered for human testing. If adverse toxicity data are obtained with the animal tests, the formulation will be eliminated from consideration, and the prospective tests on human volunteers will not be carried out. The toxicity testing program thereby serves as both a safety factor and secondary screen in the repellent development scheme.

### Description of Test

Methods of testing compounds for their potential irritancy or toxicity have become standardized over the years by the cooperative efforts of EPA, FDA, the U.S. Consumer Product Safety Commission and numerous subcommittees and Armed Forces Research departments (1-3).

A test for acute dermal toxicity evaluates the potential for systemic toxic effects of chemicals expected to come in contact with the skin. This is done by determining the median lethal dose ( $LD_{50}$ ) of a single dermal exposure to the animal species under test.

Dermal toxicity is one of the three categories of toxicity defined by route of exposure in the Federal Hazardous Substances Act (FHSA). The adult albino rabbit has been the preferred species for such reasons as size, ease of handling and restraint, and because its skin is the most permeable of all species studied. The rabbit appears to be very sensitive to dermal insult. The animal's back and abdomen are close clipped so that no less than 10% of the body surface area is available for application of material (4).

The maximum quantity of liquid test substance to be applied is 2 ml/kg. The test dose must remain in contact with the skin throughout the 24-hour exposure period. For liquids, this is assured by application of the dose inside an impermeable cuff made of plastic film. The cuff or sleeve is constructed so that the ends are reinforced and fit snugly around the trunk of the animal. The ends are tucked to permit the central portion to "balloon" and to furnish a reservoir for the dose. Such devices occlude the skin and thereby enhance penetration and potential toxicity of the test material. For this reason, routine use of occlusive dressing is not recommended unless anticipated human exposure warrants it. For materials of anticipated low toxicity, an initial range finding dose of 2 mg/kg of body weight (or approximately 2 ml/kg of body weight for a compound of similar or unknown specific gravity) applied to five or more animals of each sex with abraded skin is sufficient to demonstrate a lack of appreciable dermal toxicity. At the end of the exposure periods, any residual material is gently removed with a gauze compress, the exposed area examined at least daily for signs of systemic toxicity and localized dermal reaction. After the 14-day observation period, animals are sacrificed, a gross necropsy performed, and three sections of the exposed skin processed for histopathology (5).

### Objective of Study

The objective of this study was to determine the acute dermal toxicity potential in rats of LAIR formulation CHF1 and CHR2 (n-octyl-glutarimide).

## METHODS

### Test Substances

#### A. CHF1

CHF1 is a formulation of 50% N,N-diethyl-m-toluamide (DEET) in 25% Dow Corning 200 Fluid and 25% isopropyl alcohol. The formulation is a suspension that must be agitated to maintain continuity.

##### 1. Chemical Name: N,N-diethyl-m-toluamide

CAS: m-DEET

Molecular structure:  $C_{12}H_{17}NO$

pH: NA (non-aqueous)

Physical state: liquid, oil

Boiling point: 111 C

Compound density: 0.996 g/ml

Compound refractory index:  $n_D^{20} = 1.5212$

Compound stability: unknown

Purity: unknown (Purity at purchase was 98%, April 1979).

Manufacturer Lot No: 032697

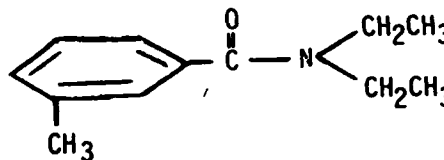
Published toxicity Data:

Oral LD<sub>50</sub> (Rat) = 200 mg/kg

Dermal LD<sub>50</sub> (Rabbit) = 3180 mg/kg

Other information:

Listed as an irritant to eyes and mucous membranes; can cause central nervous system disturbances.



##### 2. Chemical Name: Dow Corning 200 Fluid

CAS: Dimethyl siloxane polymer

Molecular structure: linear polydimethyl-siloxanes

Molecular weight: 25,000

pH: NA non-aqueous

Boiling point: unknown

Compound density: 0.971 g/ml

Compound refractory index:  $n_D^{20} = 1.4032$

Compound stability: high thermal stability - manufacturer states unlimited useful life when stored at 25 C.

Purity: unknown

Manufacturer: Dow Corning Corporation, Midland, MI 48640

Manufacturer Lot No: MA 129889

Other information:

Water repellent, low surface tension, low toxicity, essentially non-toxic and non-irritating (although temporary discomfort may result if rubbed into the eye).

3. Chemical Name: isopropanol

CAS: isopropanol

Molecular structure:  $\text{CH}_3\text{CHOHCH}_3$  ( $\text{C}_3\text{H}_8\text{O}$ )

Molecular weight: 60.09

pH: NA (non-aqueous)

Physical state: clear colorless liquid

Boiling point: 53 F (12 C)

Compound density: 0.7854 g/ml

Compound refractory index: unknown

Compound stability: unknown

Purity: unknown

Manufacturer: VWR, Scientific Products San Francisco, CA 94119

Quality Control Code: A17



Published toxicity Data:

Oral LD<sub>50</sub> (Rat) = 5840 mg/kg

Oral LD<sub>50</sub> (Dog) = 6 g/kg

Other information:

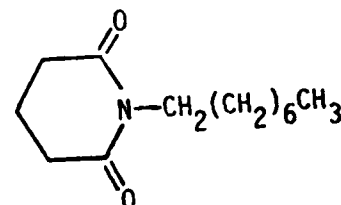
Listed as an irritant to eyes; acts as a local irritant; and, in high concentration as a narcotic. Can cause corneal burns and eye damage. Acts much like ethanol in regard to absorption metabolism and elimination but with a stronger narcotic action.

B. CHR2

Chemical Name: N-(n-octyl) glutarimide (SRI-835-39)

CAS: Unknown

Molecular structure: C<sub>13</sub>H<sub>23</sub>NO<sub>2</sub>



pH: NA (non-aqueous)

Physical state: liquid

Boiling point: 134 F (57 C)

Compound density: unknown; compound dosed estimating compound density to be close to water, with no scientific evidence to the contrary.

Compound refractory index: unknown

Compound stability: unknown

Purity: unknown

Manufacturer: SRI International, Menlo Park, CA 94025

Manufacturer Lot No: 3905H23/13

Preparation date: unknown

C. Physiologic Saline

NaCl: 0.90 g NaCl in one liter of H<sub>2</sub>O USP

Volume per container: 800 ml

Manufacturer: McGraw, Division of American Hospital Supply  
Corp, Irvine, CA 92714

Manufacturer Lot No.: JOL337AR5200

Expiration date: September 1983

Chemical data: 154 mEq/1Na, 154 mEq/1 Cl

Analytical data: none performed

D. Compound Preparation

Dr. Riefenrath of Cutaneous Hazards Division, LAIR, prepared the chemical formulation of CHF1 for the Toxicology Group. Formulation of CHF1 consists of 50% (W/V) m-DEET, 25% (W/V) Dow Corning 200 Fluid in isopropanol. Five hundred milliliters of CHF1 was prepared on 12 May 1981 (250 g of m-DEET was added to 250 g of Dow Corning 200 Fluid and the volume raised to 500 ml). CHF1 is an easily separated solution, and care must be taken to shake the bottle well, before application. CHR2 was supplied by SRI International through Mr. L. Rutledge, of Cutaneous Hazards Division. CHR2 was used in the form provided. No vehicle was used.

Animal Data

Species: Rabbit

Strain: New Zealand White

Rationale for selection: The New Zealand White Rabbit is a proven mammalian model for acute dermal studies because of its size, ease of handling, restraint and skin permeability.

Source: Elkhorn Rabbitry, 565 Starr Way, Watsonville,  
CA 95076

Pretest Conditioning:

- a. Quarantine for two weeks
- b. Animals clipped the day before dosing

Restraint: Manual restraint. Animals left their bandages alone over the 24-hour period. Some bandages

slipped in the flank area; this did not affect the systemic toxicity of the chemical in question.

Sex: Male and female.

Age: Young adult

Method of Randomization: Random Numbers Table

Animals in Each group: 5 males and 5 females per test chemical; one male and one female in saline control; one male and one female in wrapped control.

Condition of Animals at Start of Study: Normal

Mean Weight ( $\pm 1$  standard deviation) at Dosing:

2762.8 ( $\pm 158$ ) g for test animals  
3076.8 ( $\pm 140$ ) g for control

Mean Weight ( $\pm 1$  standard deviation) at Sacrifice:

2968.2 ( $\pm 163$ ) g for test animals  
3175.8 ( $\pm 290$ ) g for control

Identification Procedures: Ear labelled with laboratory indelible ink, using number system specified in SOP-OP-ARG-1

#### Environmental Conditions

Caging: Number/cage = 1; type used = stainless steel, wire mesh bottom, battery type, no bedding.

Diet: Certified Ralston Purina Rabbit Chow 5322; one carrot daily.

Water: Central line to cage battery.

Temperature: 70  $\pm 5$  F (58  $\pm 6$  C)

Relative humidity: 50  $\pm 10\%$

Photoperiod: 0530 - 2000 hr/day (light 14 1/2 hr).

#### Dosing:

**Dosing Levels:** The test was conducted as a limit test (see SOP-OP-STX-30) wherein 5 males and 5 females are assigned to each test chemical (CHF1 and CHR2). A saline control group and a "nothing applied" control group were tested after the completion of the limit test. The dose level was 2 ml/kg for both CHF1 and CHR2. According to "the state of the art" if a test is conducted at this dose level and no compound related mortality occurs, then a full study using 3 dose levels is not necessary (1). For a standard test, 10 animals per dose group would have been used -- one half of these animals would have the exposed area abraded and the other half would remain intact. (3).

**Dose volume:** According to weight; range 5.1-6.5 ml of test solution or saline.

**Duration of Exposure:** 24 hr.

**Method and Frequency of Administration:** The application sites in the abraded-skin group were abraded by use of an abrading tool designed for this experiment. It has four small metal points mounted onto a flat piece of metal that is attached to a handle. It was drawn across the area to be exposed so that only the stratum corneum was disrupted. The lines were approximately 2 cm apart over the entire exposed surface. Animals were wrapped with roll-type kling gauze (Johnson and Johnson, New Brunswick, NJ 08903, Lot 3608C228) and test material was administered using a needle-less syringe at the appropriate dose volume. The gauze was then covered with plastic wrap derived from GSA bag (#NSN8105-00-655-8285) and taped on the ends and seam with Conform<sup>R</sup> adhesive tape (Kendall Hospital Products, Boston, MA 02110, Code No. 7233). The central portion of the sleeve was allowed to "balloon" and furnish a reservoir for the chemical. The animals were observed and clinical signs recorded within six hours of administration of the test substance. The bandage was removed after 24 hours. Excess material was wiped from the area.

#### Observations:

Animals were weighed five times over the study test period. Observations were recorded twice a day (about 1700 and 1900 hr) for the limit test, once a day for the control test. At the end of the 2-week period animals were sacrificed with sodium pentobarbital and necropsied. Skin was taken from three regions and examined histopathologically (see Pathology Report).

### Historical Listing of Study Events

#### A. Limit Study

29 Apr 81	Male and female rabbits arrived at LAIR. They were checked for illness and quarantined in Room RS1409.
12 May 81	10 males and 10 females were removed from quarantine, separated into test groups and prepared for study conduction. Back, sides and abdominal hair was clipped.
13 May 81	Rabbits were dosed according to SOP-OP-STX-30. The clipped areas were abraded and test substance applied to gauze wrapped around the body. The animals were wrapped in a clear plastic and taped. Rabbits were observed frequently after dosing. Clinical signs were recorded once after dosing.
14 May 81	Bandaging materials were removed. Animals were observed twice.
15-26 May 81	Clinical observations were recorded twice a day.
27 May 81	Animals were not fed; euthanasia and necropsies were performed and several sites selected for histopathologic observation.

#### B. Control and Saline Control Study

4 Jun 81	2 males and 2 females were assigned to Saline Control or Control groups (2 groups, one of each sex), were weighed and hair clipped over the back, sides and abdomen and housed in the GLP Suite, Room RS1409.
8 Jun 81	Animals were clipped and prepared as specified in SOP-OP-STX-30 and methods.
9 Jun 81	Animals were unwrapped and observed.
10-22 Jun 81	Animals were observed once daily.
22 Jun 81	Euthanasia and necropsies were performed. Skin sites were selected for histopathologic examination.

## RESULTS

Because no compound related deaths occurred, time of death was obviously not recorded and the Dermal Lethal Dose could not be derived from this study.

### Clinical Observations

During the course of the study, observations were split into two major categories - those that applied to the general health of the animal and that which was related to skin exposure.

#### Systemic

No clinical systemic signs were noted that were interpreted as significant signs of toxicity. The CHF1 males (4 of 5) and females (4 of 5) exhibited increased respiratory rate and decreased depth during the initial observation periods. This was attributed to the skin irritation and hesitancy on the part of the animals to be touched. CHR2 exposed males (3 of 5) exhibited hunched over (humpback) attitudes and were noted to be excited (3 of 5). Other signs noted in some animals exposed to CHR2 were diarrhea, decreased feed intake, and sound production (Table 1).

#### Dermal

The most notable signs related to skin lesion were erythema, edema, scabbing, scaling, ulceration, necrosis, skin cracking, scratching, self mutilation and scaring.

Summaries by symptom and group were created from the data. To elucidate the most severe reaction that could be expected, for each clinical sign, a table was created that portrayed the maximum changes (intensity) and maximum area involved. When data was taken, location, area and intensity were graded according to a code seen at the bottom of the summary sheets. To elucidate the most probable reaction of a group of animals exposed to the chemicals, an additional table was created to demonstrate the most frequent reaction by group for each clinical sign.

Erythema, redness of the skin, was seen from the day of dosing for 4 days for CHF1 and 7 days for CHR2 along abrasions of the abdomen, flanks, sides, backs or umbilicus in 10 of 10 animals exposed to CHF1 and 10 of 10 animals exposed to CHR2 (Tables 2a and 2b). The two control rabbits exhibited erythema one day after wrapping along abrasions and taped areas. Saline exposed animals exhibited erythema for 5 to 11 days especially along the sides and abdomen (Figures 1 and for 2).

Edema was associated with the first few days of redness; 5 of 10 animals exposed to CHF1 and 5 of 10 animals to CHR2 demonstrated edema. The more severe edema was seen in animals exposed to CHR2 from less than 5 to more than 51% of the exposed area along the abrasions, flanks, sides, back and chest. Control and saline treated animals all exhibited edema over  $\leq 25\%$  of the exposed area (Tables 3a and 3b). Scab formation along abrasions and on the abdomen were seen in all groups. All animals in CHF1 and CHR2 groups demonstrated at least very slight to moderate scab formation along the abraded areas and/or abdomen (Tables 4a and 4b). Duration of scabbing from the first or second day through the 7th or 9th day was seen in all groups (Figures 1 and 2). Scaling was observed in all animals in treated groups along the abdomen, flanks, back and sides (Tables 5a and 5b). Three animals exposed to CHR2 exhibited scratching movements and lesions; one animal exposed to CHF1 exhibited scratching (Tables 6a and 6b).

Skin cracking appeared in 7 of 10 animals exposed to CHR2 especially in the females, where 5 of 5 animals demonstrated at least a slight skin cracking for 5% of the area exposed. Three of 10 animals exposed to CHF1 demonstrated skin cracking. Clearly defined but small areas of ulceration of the skin, normally occurring after removal of scab, occurred in 4 of 10 CHF1 exposed animals and 6 of 10 CHR2 animals (Tables 8a and 8b).

Subcutaneous hemorrhages were seen when bandages were removed in 2 males and 2 females exposed to CHF1 and one male exposed to CHR2. This was then later described as necrosis in the flank area. Necrosis occurred in 3 of 10 animals exposed to CHF1 and 8 of 10 exposed to CHR2 and usually involved 5% or less of the exposed area. This was seen in the flank area and was interpreted to be caused by pressure from the tape in an area of free play of skin and gravity dependency (Tables 9a and 9b).

Scarring occurred in 2 of 10 and 5 of 10 animals exposed to CHF1 and CHR2, respectively, never exceeding 5% of the exposed area (Tables 10a and 10b).

One animal in the CHF1 group demonstrated self mutilation. Three animals exposed to CHR2 were described as having defined pustule(s) equal to or less than 10% of the exposed area occurring along the abrasions and chest. One CHR2 animal was described with a defined burn in the thoracic region of less than 5% of the exposed area involved.

#### Change in Original Procedures or Protocol During Study:

1. Animals were dosed at 2 ml/kg rather than 2 g/kg.
2. Initially, three test chemicals were to be tested as a limit test for acute dermal toxicity. Insufficient N-n(n-hexyl) glutarimide

was available for use in this test. Thus, 20 animals were tested and two test chemicals were tested initially.

3. Control animals were treated in similar ways (abraded, wrapped in gauze and plastic wrap) to discuss adequately the histopathologic changes seen in the rabbit skin. Four animals (2 males, 2 females) were on hand and were assigned to control (dry, gauze) and saline (0.9% NaCl) impregnated gauze. This group was tested after the original study was terminated (see Historical Listing p.13). No control animals were requested in the original protocol, as it was a maximum limit test.

4. Animals' ears were marked with indelible ink rather than tattooed for identification. Permanent marking was not required for such a short term testing, and thus the need anesthesia required for tattooing was eliminated.

5. Temperature and relative humidity are under building-wide computer control. For most studies, we run concurrent hygrothermograph recorders. A shortage of hygrothermographs precluded the individual readings for this room for the time period of 17 May through 25 May 1981 for the limit test. Hygrothermograph chart for the control animals was recorded from 8 June through 14 June 1981.

#### Treatment of Animal Disease and Injury

Animal F8100059 exhibited a marked head tilt on 10 June 1981. This animal was in the control group and had not exhibited any skin reaction past 9 June 1981. The head tilt was possibly due to a middle ear infection and it was inhumane to continue to leave it untreated. On 16 June 1981, the veterinarian on duty administered 1 cc procaine penicillin intramuscularly (IM) and cleaned the external ear with mineral oil. On 17 June 1981, Panalog<sup>R</sup>, DMSO, and tetracaine HCl were applied to the ear and 1 cc procaine penicillin administered. On 18 June through 21 June 1981, Panalog<sup>R</sup>, DMSO, and procaine penicillin were administered. On 22 June 1981, the animal was sacrificed at the end of the experiment.

All rabbits were from the same supplier. They are not Specific Pathogen Free (SPF) or ceasarean derived and thus, endemic diseases such as ear mites, coccidiosis and nematodes are not unusual pathologic findings. If clinical signs of coccidiosis occur in quarantine, they are detained and treated with coccidiostats.

#### DISCUSSION

The acute dermal toxicity test revealed that the m-DEET formulation (CHF1) and N-(n-octyl)-glutarimide (CHR2) were not toxic at 2 ml/kg when applied to approximately 10% of the body surface of rabbits.



The application of saline to similarly prepared skin sites demonstrated definite irritation. This, in and of itself, is not unexpected. The soldier's integumentary system is hardly immune to scratches and is frequently covered by soggy clinging clothes for extended periods of time and would show irritation. The demonstration of this irritation, then, places the effects of CHF1 and CHR2 in relative terms.

Under the laboratory conditions of exposure, CHF1 and CHR2 cause mild irritation as validated by primary dermal irritation potential studies also performed on these chemicals.

In our opinion, the derivation of raw data was overkill (see clinical observations in the case of these chemicals and mountains of data were produced for molehills of effects. The obvious interpretations of these tests must also include the knowledge that rabbit skin provides "worst possible" reactions for human use evaluations.

The tape reaction problem is being researched, and methods designed to eliminate this "background noise" problem are being undertaken.

#### CONCLUSION

CHF1 and CHR2 cause mild dermal irritation to the clipped skin of rabbits exposed for 24-hour periods and observed for two weeks.

#### RECOMMENDATION

CHR1 and CHR2 on the basis of acute dermal toxicity studies and primary dermal irritation studies should be considered for continued toxicology testing.

#### REFERENCES

1. ENVIRONMENTAL PROTECTION AGENCY. Good Laboratory Practices proposed regulations (40 CFR 770, 771,772) and preamble as published in the Federal Register, 22 August 1978, 9 May 1979, 26 July and 18 April 1980 (45 FR26373)
2. FOOD AND DRUG ADMINISTRATION. Good Laboratory Practices regulations (21 CFR58) and preamble published in the Federal Register, 22 December 1978 (45 FR59986-60025)
3. INTERAGENCY REGULATORY LIAISON GROUP TESTING STANDARDS AND GUIDELINES WORK GROUP. Recommended Guidelines for Acute Dermal Toxicity Test, 1981
4. ASSOCIATION OF FOOD AND DRUG OFFICIALS OF U.S. Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (4th printing). 1959
5. COMMITTEE FOR THE REVISION OF NAS PUBLICATION 1138. COMMITTEE ON TOXICOLOGY, NATIONAL RESEARCH COUNCIL. Principles and Procedures for Evaluating the Toxicity of Household Substances. Prepared for the Consumer Product Safety Commission. Washington, DC: National Academy of Sciences, 1977

- Figure 1. GLP Study No. 81009 - Acute Dermal Toxicity of CHF1  
Average Duration of Clinical Signs in Male and  
Female Rabbit .....
- Figure 2. GLP Study No. 81009 - Acute Dermal Toxicity of CHR2  
Average Duration of Clinical Signs in Male and  
Female Rabbit .....

APPENDIX A

FIGURE 1  
GLP STUDY #81009 - ACUTE DERMAL TOXICITY OF CHF1  
AVERAGE DURATION OF CLINICAL SIGNS IN MALE AND FEMALE RABBIT

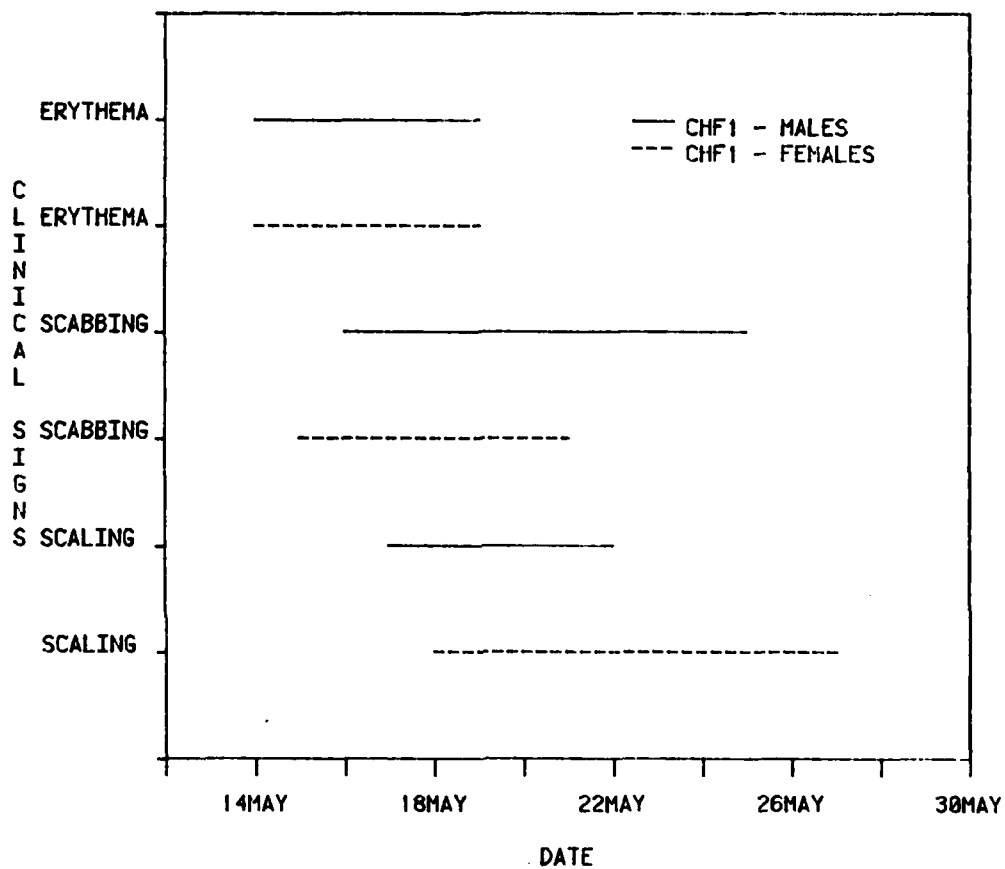


FIGURE 2  
GLP STUDY #81009 - ACUTE DERMAL TOXICITY OF CHR2  
AVERAGE DURATION OF CLINICAL SIGNS IN MALE AND FEMALE RABBIT

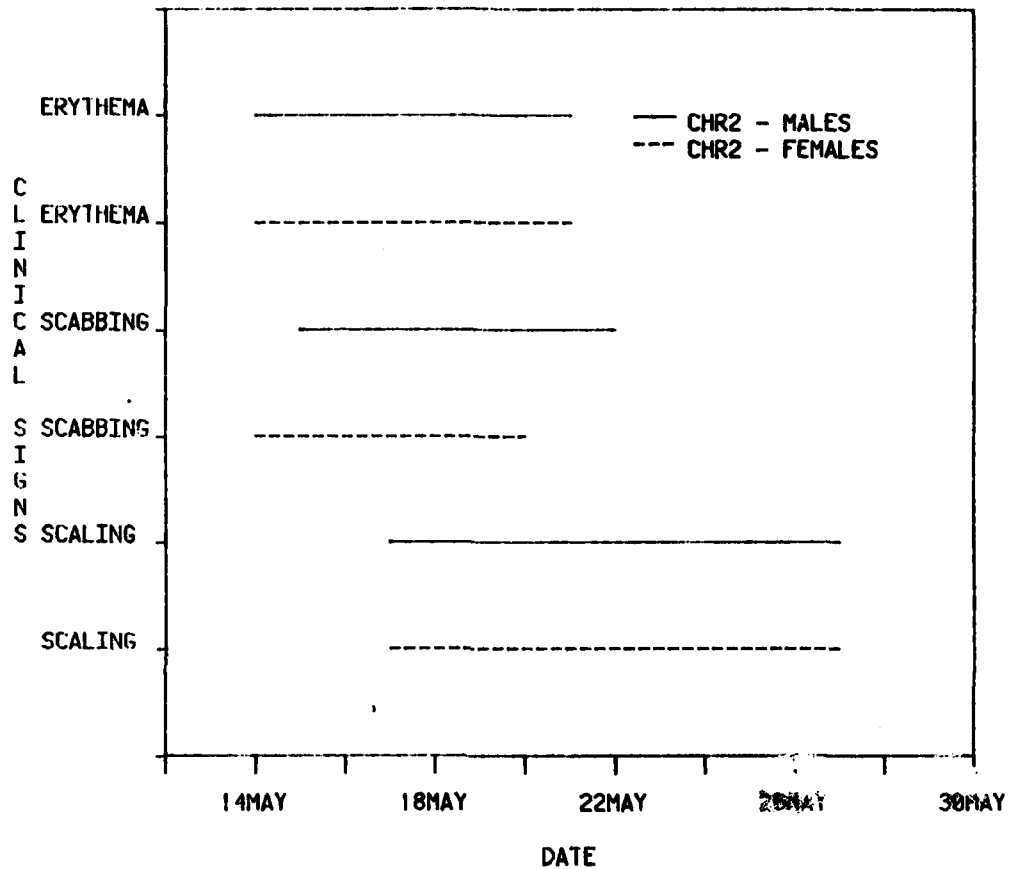


Table 1	Summary of Acute Clinical Observations
Table 2a	Erythema - Summary for Most Severe Intensity, Maximum Area, and All Locations Observed
2b	Erythema - Summary for Most Frequent Intensity, Area, and All Locations Observed
Table 3a	Edema - Summary for Most Severe Intensity, Maximum Area, and All Locations Observed
3b	Edema - Summary for Most Frequent Intensity, Area, and All Locations Observed
Table 4a	Scab Formation - Summary for Most Severe Intensity, Maximum Area, and All Locations Observed
4b	Scab Formation - Summary for Most Frequent Intensity, Area, and All Locations Observed
Table 5a	Scaling - Summary for Most Severe Intensity, Maximum Area, and All Locations Observed
5b	Scaling - Summary for Most Frequent Intensity, Area, and All Locations Observed
Table 6a	Scratching - Summary for Most Severe Intensity, Maximum Area, and All Locations Observed
6b	Scratching - Summary for Most Frequent Intensity, Area, and All Locations Observed
Table 7a	Skin Cracking - Summary for Most Severe Intensity, Maximum Area, and All Locations Observed
7b	Skin Cracking - Summary for Most Frequent Intensity, Area, and All Locations Observed
Table 8a	Ulceration - Summary for Most Severe Intensity, Maximum Area, and All Locations Observed
8b	Ulceration - Summary for Most Frequent Intensity, Area, and All Locations Observed

#### APPENDIX B

Table 9a      Necrosis - Summary for Most Severe Intensity,  
Maximum Area, and All Locations Observed

9b            Necrosis - Summary for Most Frequent Intensity,  
Area, and All Locations Observed

Table 10a     Scar - Summary for Most Severe Intensity,  
Maximum Area, and All Locations Observed

10b           Scar - Summary for Most Frequent Intensity,  
Area, and All Locations Observed

APPENDIX B

TABLE 1

## Summary of Acute Clinical Observations

(number of animals affected)  
(number of animals exposed)

CLINICAL SIGNS	CONTROL	SALINE	CHF 1		CHF 2	
			Males	Females	Males	Females
Death	0/2	0/2	0/5	0/5	0/5	0/5
Increased respiratory rate/ Decreased respiratory depth			4/5	4/5	1/5	1/5
Decreased respiratory rate			4/5	4/5	1/5	
Humpback			1/5		3/5	
Excited			1/5		3/5	
Equilibrium	1/2					
Sound production			1/5			1/5
Diarrhea			1/5	1/5		
Decreased feed intake	1/2		1/5	1/5		
Mucoid material feces		1/2				
Ear infection	1/2					



TABLE 2a

ERYTHEMA

Summary For Most Severe Intensity, Maximum Area, and All Locations Observed

Group	Sex	Total	Intensity		Area Involved		Location
			No.	Max	No.	Max	
CHF 1	M	5/5	4/5	S	3/5	25	5/5 A,0; 3/5 U,F; 2/5 S; 1/5 T
CHF 1	F	5/5	2/5	D	1/5	49	5/5 A, 0; 4/5 S; 3/5 F; 2/5 C,b,T
CHR 2	M	5/5	2/5	D	1/5	51	5/5 A; 4/5 F; 3/5 S, 2/5 b
CHR 2	F	5/5	2/5	D	1/5	51	5/5 A,F,0; 3/5 C,S,b; 2/5 0
Saline	M/F	2/2	2/2	D	1/2	51	2/2 S,A,0
Control	M/F	2/2	2/2	SL	1/2	25	2/2 taped area, 0

TABLE 2b

ERYTHEMA

Summary For Most Frequent Intensity, Area, and Location Observed

Group	Sex	Total	Intensity		Area Involved		Location
			No.	Freq	No.	Freq	
CHF 1	M	5/5	4/5	S	5/5	10	5/5 A,0
CHF 1	F	5/5	4/5	SL-M	5/5	5	5/5 A,0
CHR 2	M	5/5	5/5	SL	5/5	5-10	5/5 A
CHR 2	F	5/5	5/5	SL	5/5	25	5/5 A,F
Saline	M/F	2/2	2/2	V-D	2/2	25	2/2 S,A,0
Control	M/F	2/2	2/2	SL	1/2	5-25	2/2 taped area, 0

Severity

Exposed Area

Location

V = Very Slight  
SL = Slight  
M = Moderate  
D = Defined  
S = Severe

5 = ≤ 5%  
10 = ≤ 10%  
25 = ≤ 25%  
49 = ≤ 50%  
51 = > 50%

A = Abdomen  
B = back  
C = Thorax  
F = Flank  
S = Lateral (Side)  
O = Abrasions  
T = Test  
U = Umbilicus

GLP Study 81009  
Dermal Summary Sheet

TABLE 3a

EDEMA

Summary For Most Severe Intensity, Maximum Area, and All Locations Observed

Group	Sex	Total	Intensity No.	Max	Area Involved No.	Max	Location
CHF 1	M	3/5	1/5	S	2/5	10	3/5 U; 2/5 A,F
CHF 1	F	2/5	1/5	M	2/5	5	1/5 S,A,T,U
CHR 2	M	2/5	2/5	M	1/5	51	1/5 U, B, F
CHR 2	F	3/5	2/5	D	1/5	49	3/5 A; 2/5 S,B; 1/5 C,F,U
Saline	M/F	2/2	2/2	SL	1/2	25	2/2 S,U; 1/2 A
Control	M/F	2/2	2/2	SL	1/2	10	2/2 taped area, 1/2 U

TABLE 3b

EDEMA

Summary For Most Frequent Intensity, Area, and Location Observed

Group	Sex	Total	Intensity No.	Freq	Area Involved No.	Freq	Location
CHF 1	M	3/5	3/5	SL	3/5	5	3/5 U
CHF 1	F	2/5	1/5	V-M	2/5	5	1/5 S,A,T,U
CHR 2	M	2/5	2/5	SL-M	1/5	5-51	1/5 O,B,F
CHR 2	F	3/5	2/5	SL-D	3/5	5	3/5 A
Saline	M/F	2/2	2/2	SL	1/2	5-25	2/2 S,U
Control	M/F	2/2	2/2	SL	1/2	5-10	2/2 taped area

Severity

Exposed Area

Location

V = Very Slight  
SL = Slight  
M = Moderate  
D = Defined  
S = Severe

5 =  $\leq$  5%  
10 =  $\leq$  10%  
25 =  $\leq$  25%  
49 =  $\leq$  50%  
51 =  $>$  50%

A = Abdomen  
B = Back  
C = Thorax  
F = Flank  
S = Lateral (Side)  
O = Abrasions  
T = Test  
U = Umbilicus

TABLE 4a  
SCAB FORMATION

Summary For Most Severe Intensity, Maximum Area, and All Locations Observed

Group	Sex	Total	Intensity No.	Max	Area Involved No.	Max	Location
CHF 1	M	5/5	2/5	D	2/5	10	5/5 A,0; 3/5 F; 1/5 L,U,B,HL
CHF 1	F	5/5	1/5	D	1/5	25	5/5 A,0; 4/5 S; 3/5 F; 1/5 B,U
CHR 2	M	5/5	5/5	M	1/5	25	5/5 O,A; 3/5 F; 1/5 B,U
CHR 2	F	5/5	1/5	D	2/5	25	5/5 A,F; 3/5 O; 2/5 S; 1/5 B
Saline	M/F	2/2	1/2	D	2/2	5	2/2 O,A
Control	M/F	1/2	1/2	V	1/2	5	1/2 O,U

TABLE 4b  
SCAB FORMATION

Summary For Most Frequent Intensity, Area, and Location Observed

Group	Sex	Total	Intensity No.	Freq	Area Involved No.	Freq	Location
CHF 1	M	5/5	4/5	V	5/5	5	5/5 A,0
CHF 1	F	5/5	5/5	V	4/5	5	5/5 A,0
CHR 2	M	5/5	5/5	SL-M	5/5	5	5/5 A,0
CHR 2	F	5/5	4/5	SL	3/5	10	5/5 A,F
Saline	M/F	2/2	2/2	SL	2/2	5	2/2 O,A
Control	M/F	1/2	1/2	V	1/2	5	1/2 O,A,U

Severity

V = Very Slight  
SL = Slight  
M = Moderate  
D = Defined  
S = Severe

Exposed Area

5 = < 5%  
10 = < 10%  
25 = < 25%  
49 = < 50%  
51 = > 50%

Location

A = Abdomen  
B = back  
C = Thorax  
F = Flank  
S = Lateral (Side)  
O = Abrasions  
T = Test  
U = Umbilicus  
HL = Hindleg

GLP Study 81009  
Dermal Summary Sheet

TABLE 5a  
SCALING

Summary For Most Severe Intensity, Maximum Area, and All Locations Observed

Group	Sex	Total	Intensity		Area Involved		Location
			No.	Max	No.	Max	
CHF 1	M	5/5	5/5	SL	2/5	10	5/5 A,U; 4/5 B,S; 3/5 T
CHF 1	F	5/5	1/5	M	2/5	25	4/5 A,U; 3/5 S,F; 1/5 C,b
CHR 2	M	5/5	2/5	D	1/5	51	5/5 A; 4/5 S,F; 3/5 B; 2/5 U
CHR 2	F	5/5	2/5	D	3/5	51	5/5 A,S; 4/5 F,b; 3/5 U
Saline	M/F	0/2					
Control	M/F	0/2					

TABLE 5b  
SCALING

Summary For Most Frequent Intensity, Area, and Location Observed

Group	Sex	Total	Intensity		Area Involved		Location
			No.	Freq	No.	Freq	
CHF 1	M	5/5	5/5	V-SL	5/5	5	5/5 A,O
CHF 1	F	5/5	5/5	V-SL	4/5	5	5/5 F,O
CHR 2	M	5/5	5/5	SL	5/5	25	5/5 A
CHR 2	F	5/5	5/5	M	5/5	10-25	5/5 A,S
Saline	M/F	0/2					
Control	M/F	0/2					

Severity

Exposed Area

Location

V = Very Slight  
SL = Slight  
M = Moderate  
D = Defined  
S = Severe

5 =  $\leq$  5%  
10 =  $\leq$  10%  
25 =  $\leq$  25%  
49 =  $\leq$  50%  
51 =  $>$  50%

A = Abdomen  
B = Back  
C = Thorax  
F = Flank  
S = Lateral (Side)  
O = Abrasions  
T = Test  
U = Umbilicus

TABLE 6a  
SCRATCHING

Summary For Most Severe Intensity, Maximum Area, and All Locations Observed

Group	Sex	Total	Intensity		Area Involved		Location
			No.	Max	No.	Max	
CHF 1	M	0/5					
CHF 1	F	1/5	1/5	M	1/5	10	1/5 S
CHR 2	M	2/5	2/5	SL	2/5	5	2/5 C; 1/5 S
CHR 2	F	1/5	1/5	SL	1/5	5	1/5 C
Saline	M/F	0/2					
Control	M/F	0/2					

TABLE 6b  
SCRATCHING

Summary For Most Frequent Intensity, Area, and Location Observed

Group	Sex	Total	Intensity		Area Involved		Location
			No.	Freq	No.	Freq	
CHF 1	M	0/5					
CHF 1	F	1/5	1/5	M	1/5	5-10	1/5 S
CHR 2	M	2/5	2/5	SL	2/5	5	2/5 C
CHR 2	F	1/5	1/5	SL	1/5	5	1/5 C
Saline	M/F	0/2					
Control	M/F	0/2					

Severity	Exposed Area	Location	
V = Very Slight	5 = ≤ 5%	A = Abdomen	O = Abrasions
SL = Slight	10 = ≤ 10%	B = Back	T = Test
M = Moderate	25 = ≤ 25%	C = Thorax	U = Umbilicus
D = Defined	49 = ≤ 50%	F = Flank	
S = Severe	51 = > 50%	S = Lateral (Side)	

TABLE 7a  
SKIN CRACKING

Summary For Most Severe Intensity, Maximum Area, and All Locations Observed

Group	Sex	Total	Intensity		Area Involved		Location
			No.	Max	No.	Max	
CHF 1	M	1/5	1/5	M	1/5	5	1/5 F
CHF 1	F	2/5	1/5	D	2/5	5	1/5 F,S
CHR 2	M	2/5	2/5	M	1/5	51	1/5 B,A,S
CHR 2	F	4/5	1/5	D	1/5	51	4/5 B; 2/5 S,F; 1/5 A
Saline	M/F	0/2					
Control	M/F	0/2					

TABLE 7b  
SKIN CRACKING

Summary For Most Frequent Intensity, Area, and Location Observed

Group	Sex	Total	Intensity		Area Involved		Location
			No.	Freq	No.	Freq	
CHF 1	M	1/5	1/5	SL-M	1/5	5	1/5 F
CHF 1	F	2/5	1/5	SL-D	2/5	5	1/5 F,S
CHR 2	M	2/5	2/5	V-M	2/5	5-25	1/5 B,A,S
CHR 2	F	4/5	3/5	M	4/5	5	4/5 B
Saline	M/F	0/2					
Control	M/F	0/2					

Severity	Exposed Area	Location	
V = Very Slight	5 = ≤ 5%	A = Abdomen	O = Abrasions
SL = Slight	10 = ≤ 10%	B = Back	T = Test
M = Moderate	25 = ≤ 25%	C = Thorax	U = Umbilicus
D = Defined	49 = ≤ 50%	F = Flank	
S = Severe	51 = > 50%	S = Lateral (Side)	

TABLE 8a  
ULCERATION

Summary For Most Severe Intensity, Maximum Area, and All Locations Observed

Group	Sex	Total	Intensity No. Max	Area Involved No. Max	Location
CHF 1	M	2/5	2/5 M	2/5 5	2/5 F; 1/5 S
CHF 1	F	2/5	2/5 M	1/5 10	1/5 C,A,F,S
CHR 2	M	3/5	2/5 D	3/5 5	1/5 F,A,S
CHR 2	F	3/5	1/5 D	3/5 5	2/5 F; 1/5 B,S
Saline	M/F	0/2			
Control	M/F	0/2			

TABLE 8b  
ULCERATION

Summary For Most Frequent Intensity, Area, and Location Observed

Group	Sex	Total	Intensity No. Freq	Area Involved No. Freq	Location
CHF 1	M	2/5	2/5 M	2/5 5	2/5 F
CHF 1	F	2/5	2/5 M	2/5 5	1/5 C,A,F,S
CHR 2	M	3/5	2/5 SL	3/5 5	1/5 F,A,S
CHR 2	F	3/5	1/5 SL-D	2/5 5	2/5 F
Saline	M/F	0/2			
Control	M/F	0/2			

Severity

V = Very Slight  
SL = Slight  
M = Moderate  
D = Defined  
S = Severe

Exposed Area

5 = < 5%  
10 = < 10%  
25 = < 25%  
49 = < 50%  
51 = > 50%

Location

A = Abdomen U = Abrasions  
B = Back T = Test  
C = Thorax U = Umbilicus  
F = Flank  
S = Lateral (Side)

GLP Study 81009  
Dermal Summary Sheet

TABLE 9a

NECROSIS

Summary For Most Severe Intensity, Maximum Area, and All Locations Observed

Group	Sex	Total	Intensity		Area Involved		Location
			No.	Max	No.	Max	
CHF 1	M	2/5	2/5	S	1/5	25	2/5 F,A
CHF 1	F	1/5	1/5	M	1/5	5	1/5 A,F
CHR 2	M	3/5	1/5	D	3/5	5	2/5 F; 1/5 A,U
CHR 2	F	5/5	1/5	D	5/5	5	5/5 A; 3/5 U; 2/5 F
Saline	M/F	0/2					
Control	M/F	0/2					

TABLE 9b

NECROSIS

Summary For Most Frequent Intensity, Area, and Location Observed

Group	Sex	Total	Intensity		Area Involved		Location
			No.	Freq	No.	Freq	
CHF 1	M	2/5	2/5	D	2/5	5-10	2/5 F,A
CHF 1	F	1/5	1/5	SL-M	1/5	5	1/5 A,F
CHR 2	M	3/5	1/5	SL-U	3/5	5	2/5 F
CHR 2	F	5/5	3/5	SL	5/5	5	5/5 A,U
Saline	M/F	0/2					
Control	M/F	0/2					

Severity

V = Very Slight  
SL = Slight  
M = Moderate  
D = Defined  
S = Severe

Exposed Area

5 = ≤ 5%  
10 = ≤ 10%  
25 = ≤ 25%  
49 = ≤ 50%  
51 = > 50%

Location

A = Abdomen      O = Abrasions  
B = Back          T = Test  
C = Thorax        U = Umbilicus  
F = Flank  
S = Lateral (Side)



TABLE 10a

SCAR

Summary For Most Severe Intensity, Maximum Area, and All Locations Observed

Group	Sex	Total	Intensity		Area Involved		Location
			No.	Max	No.	Max	
CHF 1	M	1/5	1/5	M	1/5	5	1/5 F
CHF 1	F	1/5	1/5	SL	1/5	5	1/5 S
CHR 2	M	2/5	1/5	M	2/5	5	2/5 F
CHR 2	F	3/5	2/5	SL	3/5	5	2/5 F; 1/5 S
Saline	M/F	0/2					
Control	M/F	0/2					

TABLE 10b

SCAR

Summary For Most Frequent Intensity, Area, and Location Observed

Group	Sex	Total	Intensity		Area Involved		Location
			No.	Freq	No.	Freq	
CHF 1	M	1/5	1/5	M	1/5	5	1/5 F
CHF 1	F	1/5	1/5	V-SL	1/5	5	1/5 S
CHR 2	M	2/5	1/5	V-M	2/5	5	2/5 F
CHR 2	F	3/5	2/5	V-SL	3/5	5	2/5 F
Saline	M/F	0/2					
Control	M/F	0/2					

Severity

Exposed Area

Location

V = Very Slight  
SL = Slight  
M = Moderate  
D = Defined  
S = Severe

5 = < 5%  
10 = < 10%  
25 = < 25%  
49 = < 50%  
51 = > 50%

A = Abdomen  
B = Back  
C = Thorax  
F = Flank  
S = Lateral (Side)  
O = Abrasions  
T = Teat  
U = Umbilicus

Pathology Summary .....

Table I - Gross Lesions in the Skin of Rabbits  
Exposed to CHR1 and CHR2 .....

Table II - Microscopic Lesions of Rabbits Exposed  
to CHF1 and CHR2 .....

Pathology Summary and Interpretation of GLP Study 81-000,  
Acute Dermal Toxicity Study of CHF1\* and CHR2\*\*

Compound CHF1 was applied to shaved abraded skin of 5 male and 5 female New Zealand white rabbits at a dosage rate of 2 ml/kg (Group I). Group II consisted of 5 male and 5 female rabbits exposed to 2 ml/kg of CHR2. Gauze bandages were used to hold the test material in place for 24 hours. Animals were observed for 14 days after application of the test material. Two control groups were used. For bandage controls, the shaved, abraded skin of 1 male and 1 female rabbit was bandaged for 24 hours and observed for 14 days. For saline controls, the shaved, abraded skin of 1 male and 1 female rabbit was exposed to physiological saline, bandaged for 24 hours and observed for 14 days.

There were no gross skin lesions in the two male and two female control rabbits. Gross skin lesions in experimental groups are tabulated in Table I and consisted of surface flakes and scales, abrasions, scabs, and healed scars. The latter three lesions appeared to be due to irritation by the gauze bandages and trauma from abrasions produced prior to application of the test compound. Surface flakes and scales were present in 1/5 Group I females, 3/5 Group II females and 2/5 Group II males. This change might be caused by the test compounds, however, it occurred in only 5/10 of the animals exposed to CHR2 and only 1/10 animals exposed to CHF1. The exact nature of this change is unknown. It may indicate increased rate of keratinization and desquamation of the surface epithelium. There was no corresponding histologic change in affected skin specimens probably because the flakes and scales were not tightly adherent to the surface and may have washed off during tissue processing. No microscopic changes were recognized in the epithelium or dermis of these specimens that could be attributed to application of the test compound.

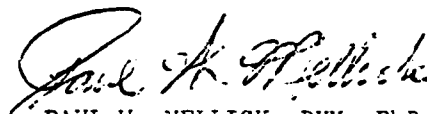
Microscopic lesions in the skin of exposed sites are tabulated for each animal in Table II. The most common change was infiltration of the superficial dermis with heterophils, macrophages, lymphocytes, and plasma cells. This lesion was present in most of the skin specimens examined (including those from control animals) and was usually of minimal intensity. Several animals had fibrous connective tissue proliferation in addition to mixed inflammatory cell infiltration. In only one specimen of the 72 skin sections examined in this study was there ulceration and this was a very small focal lesion. All these changes were probably caused by intentional abrasion and bandage irritation.

\*CHF1 - 50% DEET + 25% Dow Corning Fluid 200 + 25% Isopropanol

\*\*CHR2 - N (n-octyl) glutaramide

A few gross lesions were observed in tissues other than exposed skin. The most common were small yellowish-white foci in the liver that are typical of lesions caused by Eimeria stiedae, a coccidia that infects intrahepatic bile ducts of rabbits. These lesions were present in 2/4 controls, 3/5 Group I females, 2/5 Group I males, 2/5 Group II females, and 1/5 Group II males. Two rabbits had crusty flakes and scabs in both ears typical of infestation with Psoroptes cuniculi, the ear mite of rabbits. Other incidental lesions considered unrelated to the application of test compounds were an abscess in the thymus in 1/5 Group I female, an undescended testes in 1/5 Group I male, and congestion of the trachea and abscessation of the cecal diverticulum in 1/5 Group II female.

In summary, application of compound CHF1 under gauze bandage to the skin of New Zealand White Rabbits for 24 hours caused no gross or microscopic lesions in the skin where it was applied and no gross lesions in any of the other organs and tissues after 14 days. Application of CHR2 under these conditions may have caused surface flakes and scales on the skin of half the exposed animals. This change could indicate increased rate of epithelial keratinization and desquamation. There were no microscopic correlates to this gross observation in the skin sections examined. There were no gross lesions observed in other organs or tissues of rabbits attributable to CHR2.



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TABLE I  
Gross Lesions in the Skin of Rabbits  
Exposed to CHF1 and CHR2

EXPERIMENTAL GROUP	SURFACE FLAKES AND SCALES	ABRASIONS	SCABS	HEALED SCARS
Controls - 2 males & 2 females	0/4	0/4	0/4	0/4
Group I Females	1/5	1/5	0/5	2/5
Group I Males	0/5	0/5	1/5	4/5
Group II Females	3/5	0/5	0/5	1/5
Group II Males	2/5	0/5	0/5	1/5

### Explanation of Table II

Mixed inflammatory cell infiltrates consisted of heterophils, macrophages, lymphocytes and plasma cells in the superficial dermis. Fibrosis designates proliferation of fibroblasts and was seen only in the superficial dermis usually near the mixed inflammatory cells. Ulceration designates the absence of the epidermal layer with intense infiltration of inflammatory cells in the subjacent dermis.

0 = Lesion not present

1 = Minimal change

2 = Mild change

3 = Moderate change

4 = Severe change

VA = Skin from ventral abdomen exposure site.

DL = Skin from dorsal lumbar exposure site.

F = Skin from flank exposure site.

TABLE II  
Microscopic Lesions in Skin of Rabbits Exposed to CHF1 or CHR2

	MIXED INFLAMMATORY CELL INFILTRATE, DERMIS	FIBROSIS	ULCERATION
CONTROL GROUP			
F8100047 (female)			
VA	1	0	0
DL	1	0	0
F	1	0	0
F8100058 (male)			
VA	0	0	0
DL	1	1	0
F	1	0	0
F8100065 (female)			
VA	1	0	0
DL	1	1	0
F	1	1	0
F8100035			
VA	1	0	0
DL	0	0	0
F	0	0	0
GROUP 1 FEMALES			
F8100064			
VA	0	0	0
DL	0	0	0
F	0	0	0
F8100068			
VA	2	2	0
DL	1	0	0
F	2	0	0
F8100069			
VA	0	0	0
DL	0	0	0
F	1	1	0
F8100071			
VA	2	0	2
DL	1	0	0
F	0	0	0
F8100072			
VA	1	0	0
DL	1	0	0
F	1	0	0

Table II (cont.)

	MIXED INFLAMMATORY CELL INFILTRATE, DERMIS	FIBROSIS	ULCERATION
GROUP I MALES			
F8100033			
VA	0	0	0
DL	1	0	0
F	1	1	0
F8100034			
VA	0	0	0
DL	2	0	0
F	1	0	0
F8100036			
VA	2	2	0
DL	2	0	0
F	1	0	0
F8100037			
VA	1	0	0
DL	1	0	0
F	0	0	0
F8100038			
VA	0	0	0
DL	1	0	0
F	3	3	0
Note: Mild acanthosis of epithelium was also present			
GROUP II FEMALES			
F8100063			
VA	1	1	0
DL	2	0	0
F	2	0	0
F8100066			
VA	1	0	0
DL	1	0	0
F	1	0	0
F8100067			
VA	1	0	0
DL	2	2	0
F	1	0	0
F8100070			
VA	1	0	0
DL	1	0	0
F	1	0	0
F8100073			
VA	2	2	0
DL	1	0	0
F	0	0	0



Table II (cont.)

	MIXED INFLAMMATORY CELL INFILTRATE, DERMIS	FIBROSIS	ULCERATION
GROUP II MALES			
F8100035			
VA	2	2	0
DL	1	0	0
F	0	0	0
F8100039			
VA	1	0	0
DL	1	0	0
F	1	0	0
F8100040			
VA	0	0	0
DL	1	0	0
F	1	0	0
F8100041			
VA	0	0	0
DL	1	0	0
F	1	0	0
F8100074			
VA	1	0	0
DL	1	0	0
F	1	0	0

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